

A System for Characterizing Reproductive and Diapause Morphology in the Boll Weevil (Coleoptera: Curculionidae)

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ABSTRACT Diapause in the boll weevil, *Anthonomus grandis* Boheman, has been extensively studied, but remains poorly understood. In particular, results of diapause induction studies have been inconsistent, and the body of published literature does not clearly indicate the factors or cues responsible for induction. A likely contributor to this variability may be the differences among studies in the criteria used to distinguish diapause. We present illustrated descriptions of the stages of male and female reproductive development, and of the fat body types and conditions associated with reproductive and diapausing weevils. These descriptions provide clearer and more extensive guidelines for the classification of reproductive and diapausing boll weevils than were previously available. We also propose criteria for distinguishing diapause based on specific characters of the fat body and male and female reproductive organs. These criteria provide a means of standardizing diapause assessments in future studies, or at least a baseline for comparison when describing alternate criteria, and should serve to clarify these descriptions in future reports.

KEY WORDS *Anthonomus grandis*, boll weevil, reproductive morphology, diapause

A KEY ASPECT OF THE ECOLOGY of the boll weevil, *Anthonomus grandis* Boheman, that facilitated its spread throughout most of the United States Cotton Belt is its ability to survive temperate winters. It is widely recognized that the adult boll weevil overwinters in temperate regions in a state of dormancy. Brazzel and Newsom (1959) characterized this dormancy as a reproductive diapause based on its association with hypertrophy of the fat body, atrophy of the reproductive organs, and reductions in respiration rate and body water content. Since this initial report of diapause, numerous investigators have examined the factors inducing the dormancy and its ecological and physiological consequences. Yet, this facet of boll weevil ecology remains poorly understood.

The body of literature regarding the induction of diapause in the boll weevil is extensive, but does not clearly indicate the causative factors or cues. Most investigations have focused on the influences of photoperiod. For example, Earle and Newsom (1964), Lloyd et al. (1967), Mangum et al. (1968), and Sterling (1972) reported diapause responses to various photoperiods, but their results were not entirely consistent. More recently, Wagner and Villavaso (1999a) reported a model of the seasonal incidence of diapause

based on the presumption that diapause was primarily induced by exposure to decreasing day lengths. Earle and Newsom (1964) and Lloyd et al. (1967) found that low nighttime temperatures also played a role in diapause induction. Furthermore, Lloyd et al. (1967) reported that when held on intact cotton plants (*Gossypium hirsutum* L.) under any of four photoperiods, weevils tended to enter diapause when primarily fruit (bolls) were present and became reproductive when plants were producing flower buds (squares). However, plant phenology did not effectively mask the influences of low nighttime temperatures. In preliminary reports, Spurgeon and Raulston (1998a) and Spurgeon and Esquivel (2000) found the diapause response could be controlled by adult diet irrespective of photoperiod or temperature. Still other investigators (Cobb and Bass 1968, Wagner et al. 1999) have reported that diapause induction is dependent on combinations of photoperiod, temperature, or diet.

Even the developmental stage(s) receiving the induction cues has been subject to debate. Lloyd et al. (1967), Mangum et al. (1968), Spurgeon and Raulston (1996, 1998a), and Spurgeon and Esquivel (2000) induced diapause in the adult stage. Lloyd et al. (1967) found the larval stage was also sensitive to induction cues. Mangum et al. (1968) indicated the diapause response could be influenced in the egg stage, and Earle and Newsom (1964) reported diapause was induced in the egg and larval stages. Sterling (1972) reported that all stages were sensitive to photoperiod,

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including the parental adults. Finally, Wagner et al. (1999) reported all stages were sensitive to induction cues, but that individuals remained sensitive for different durations.

Spurgeon and Raulston (1998b) suggested some of the differences among results of previous diapause induction studies may be accounted for by the feeding regimes typically used, along with the general failure to account for differences in physiological ages among weevils held at different temperatures. Spurgeon and Raulston (1998a) further suggested that some of the differences among reports may have resulted from inconsistent application of the criteria used to distinguish diapause. Betz and Lambremont (1967) and Cole and Adkisson (1983) report using the criteria of Brazzel and Newsom (1959). Lloyd and Merkl (1961) added a third category of intermediate diapause, distinguished from categories of firm diapause or reproductive by the presence of some eggs and some visible fat in females and testes reduced in size, but larger than 0.6 mm in diameter in males. Categories of intermediate and firm diapause were combined in their results, while Lloyd et al. (1964) reported results for intermediate and firm diapause classes separately. Mitchell and Mistic (1965), McCoy et al. (1968), and Tingle et al. (1971) used these criteria and pooled intermediate and firm diapause in their results. Tingle and Lloyd (1969) used moisture and fat content in addition to these criteria in one experiment, but used only moisture and fat content in a second experiment. Sivasupramaniam et al. (1995) used criteria that differed from those of Lloyd and Merkl (1961) in that reproductive status was given greater weight than fat body condition in determination of the firm diapause category. However, the basis and procedure for weighting were not described. Earle and Newsom (1964), Harris et al. (1967), Cobb and Bass (1968), Mangum et al. (1968), and Graham et al. (1979) based diapause assessments on some combination of fat body size and gonadal size and color, but do not describe the characters used in these assessments. Mitlin and Wiygul (1976) used gonadal and fat body characters to assess diapause in a dissected subsample, but used only estimates of fat content viewed through the cuticle for weevils used in biochemical analyses. Lambremont (1961) and Guerra et al. (1982) based diapause classifications solely on observations of fat bodies through the dorsal cuticle, while Lambremont et al. (1964), Carter and Phillips (1973), and Thompson and Leggett (1978) considered only the condition of the reproductive organs. Wagner and Villavaso (1999a, 1999b) used a system of distinguishing prediapause that was unique to their studies. However, they do not describe the characteristics of the six fat body categories they used nor the rationale for the combinations of characters to distinguish prediapausing from reproductive weevils. Furthermore, they provided an additional classification of undetermined, and excluded those weevils from analyses.

Regardless of the criteria used to distinguish diapause, most previous reports focus exclusively on the apparent amounts of fat body, the sizes of reproduc-

tive organs, or both, with little or no consideration for distinct characteristics of those tissues. During our studies of reproductive development (Spurgeon and Raulston 1996, 1997, 1998b; Suh and Spurgeon 2002), diapause responses to feeding regimes (Spurgeon and Raulston 1998a, Spurgeon and Esquivel 2000), overwintering survival and emergence patterns (Westbrook et al. 2001, Spurgeon 2002), hemolymph supercooling (Suh et al. 2003), and pheromone production (Spurgeon 2001, Young and Spurgeon 2002), we observed characteristics of reproductive and diapause development either not described or not adequately illustrated in previous reports. In this work, we illustrate these morphological characters with the intent of providing a point of reference for subsequent descriptions of criteria for distinguishing between reproductive and diapausing boll weevils.

Materials and Methods

Adult weevils were reared from infested squares collected from the plants in commercial cotton fields near Weslaco, Texas. Squares were held in screened cages in an environmental chamber maintained at $29.4 \pm 1^\circ\text{C}$ with a 13:11 (L:D) h photoperiod. Beginning ≈ 5 d after square collection, squares were examined daily for the presence of pupae. Pupae were transferred to disposable 100×15 -mm petri plates, in which they were held in groups of 35–50 on a layer of moistened vermiculite. Pupal plates were also held in the environmental chamber and were examined daily for newly eclosed adults. Newly eclosed adults were sexed using the method of Sappington and Spurgeon (2000) and partitioned among feeding regimes to produce the desired morphological characters.

Characteristics of juvenile fat (retained from the pupal stage) were documented on the day of adult eclosion and the following day. Weevils intended for illustration of the stages of reproductive development were held individually in 100×15 -mm petri plates and were supplied a fresh square (6–9-mm diameter) with bracteoles intact each day as well as a short (≈ 1 -cm) section of cotton dental wick saturated with deionized water, as described by Spurgeon and Raulston (1998b). Weevils intended to illustrate diapause characters were held in mixed-sex groups of 12 ♂♂ and 13 ♀♀ in 473-cm^3 cardboard cartons, as described by Spurgeon and Raulston (1998a). These weevils were fed 6–9-mm diameter squares, with bracteoles removed at a rate of one square per five weevils. Squares were replaced daily. The weevils were also supplied water in a 29.5-ml plastic cup with a cotton wick extending through the lid. Weevils assigned to either reproduction- or diapause-inducing diets were held under the same conditions as the infested squares. Weevils not assigned to the reproduction- and diapause-inducing diets were pooled in a screened cage and held in the laboratory. This group of mixed-sex and mixed-age weevils was fed a more limited diet of squares with bracts removed. Squares were typically replaced twice or thrice weekly. These conditions were used to produce female weevils whose ovaries

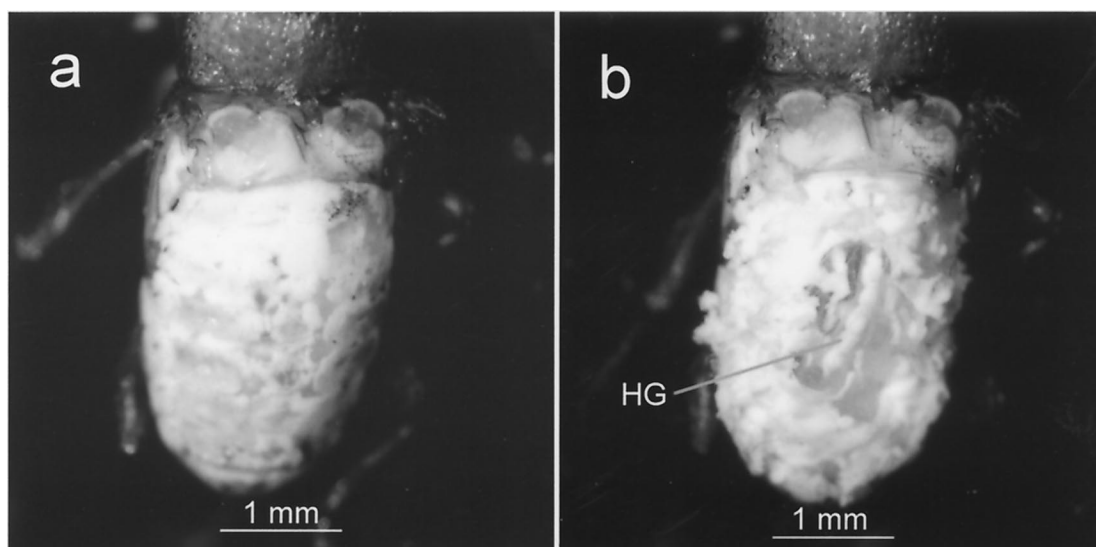


Fig. 1. Juvenile fat retained from the pupal stage by a newly eclosed boll weevil adult. (a) Viewed through the cuticle. (b) With the cuticle removed. HG, hindgut.

illustrated developmental stasis or oosorption and to obtain males whose seminal vesicles were refilling after repeated matings.

Weevils fed the reproductive diet were dissected at ages ranging from 0 to 9 d old. Weevils fed the diapause-inducing diet and those held in the laboratory were mostly between 12 and 17 d old when dissected, but some were >17 d of age. Dissections illustrating fat bodies, male reproductive tracts, and isolated organs were conducted in a paraffin-lined dish under a dissecting Ringers. Dissections illustrating intact female reproductive tracts were performed under deionized water to reduce the frequency of vigorous muscular contractions that interfered with efforts to obtain adequate photographs. Dissected weevils were anchored to the paraffin by an insect pin inserted through the prothorax. Elytra and wings were removed using forceps, and tissues were exposed for examination by grasping the abdominal cuticle at its anterior margin and pulling it up and rearward while stabilizing the metathorax with a second pair of forceps. Photographs were taken with Plus-X Pan ISO 125 black and white film (Eastman Kodak, Rochester, NY) at magnifications of 12.5–63 \times using an Olympus SZ60 dissecting microscope with a PM-PBK-3 automatic exposure 35-mm camera attachment (Olympus America, Melville, NY).

Terminology used to describe the illustrations were adapted from Burke (1959), Chadbourne (1961), and Grodowitz and Brewer (1987). Descriptions of the stages of reproductive and diapause development are derived from Spurgeon and Raulston (1998a, b) combined with our experience from additional study.

Results and Discussion

Fat Body Types and Conditions. A distinctive fat body type, referred to as juvenile fat by Spurgeon and

Raulston (1998b), is carried over from the pupal stage to the teneral adult. The dorsal abdominal cuticle of the teneral adult is less pigmented than that of older counterparts, making the juvenile fat readily visible in the intact weevil (Fig. 1a). The juvenile fat (Fig. 1b) is slightly gray and of very fine texture relative to other fat body types (see below). Based on visual estimates, abundance of fat at eclosion commonly varies from present, but obscuring only a small portion of the other internal organs, to abundant and obscuring most of those organs. In our experience, boll-fed larvae tend to produce adults with a greater complement of juvenile fat than do square-fed larvae (D.W.S., unpublished data). These observations are consistent with those of Walker and Shipp (1963), who reported that boll weevil pupae removed from bolls contained markedly more fat than those reared on artificial diet. The juvenile fat is present for a short time, ranging from 1 to 2 d at temperatures $\geq 29.4^{\circ}\text{C}$ to 15–20 d at 12.8°C (Spurgeon and Raulston 1998b), and assumes a more diffuse and granular appearance at the later ages (Fig. 2a).

The fat body type typically associated with a high degree of reproductive development is translucent to opaque, grayish in color, and sheet-like in conformation (Fig. 2b). It can vary in abundance from barely detectable to nearly completely obscuring other organs, and appears more opaque when present in larger quantities. In our experience, perception of its abundance is influenced by the degree of reproductive development and the contents of the hindgut in that it appears a more dominating feature of the abdominal cavity when the reproductive organs are small and the hindgut is empty.

The fat body type associated with dormancy is predominantly bright white and composed of distinct globules (Fig. 2, c and d). In some individuals, a yellow fat is present, but is never predominant, although the

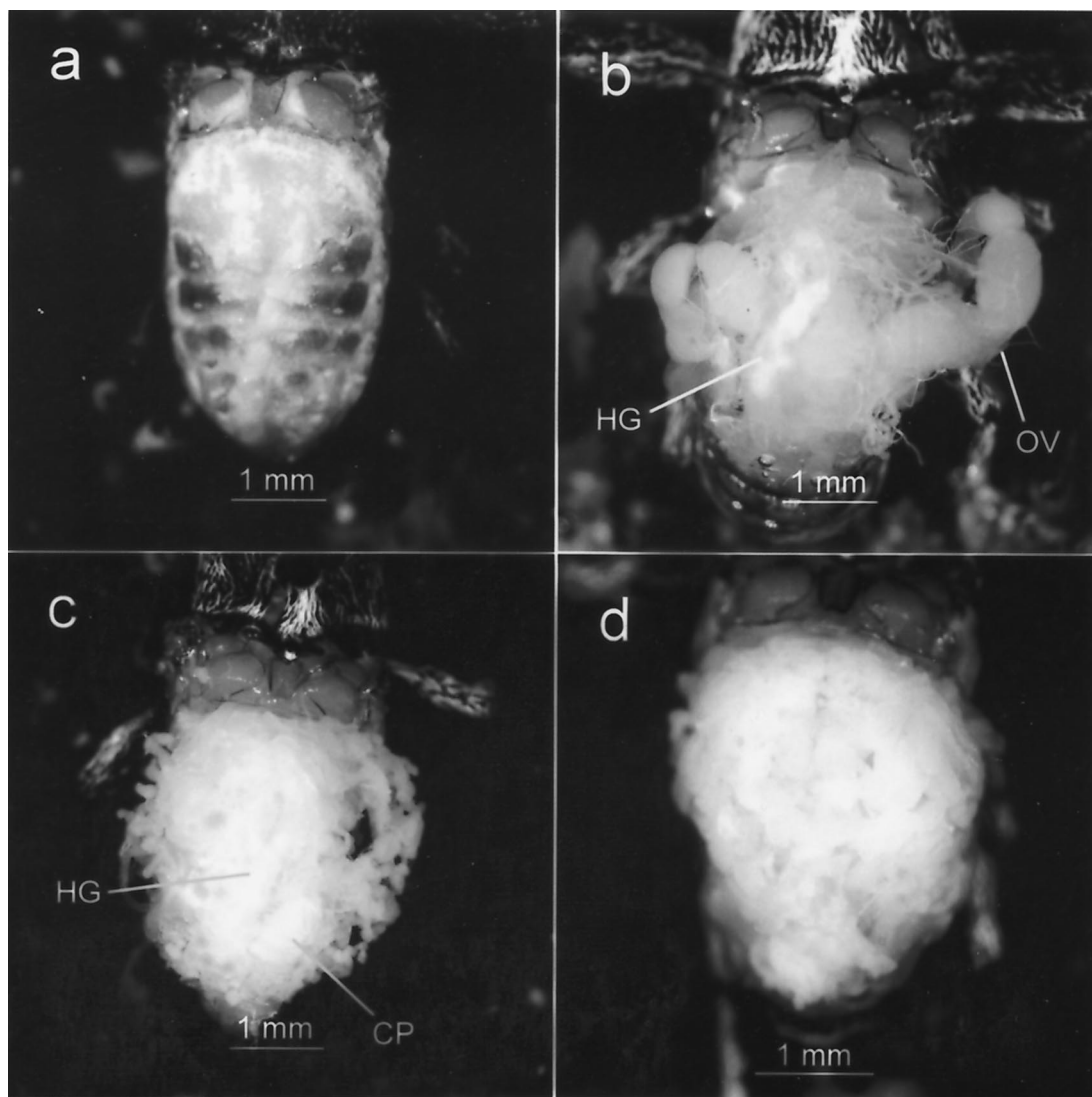


Fig. 2. Fat body types and conditions representative of reproductive and diapausing boll weevil adults. (a) Remnants of juvenile fat viewed through the cuticle of a 1-d-old weevil. (b) Translucent and sheet-like fat in a reproductive female weevil. (c) Dormant-type fat body composed of well-formed globules and rating intermediate because portions of the alimentary and reproductive tracts at the posterior of the abdomen are visible. (d) Dormant-type fat body composed of well-formed globules and rating fat because other internal organs are completely or nearly completely obscured. CP, copulatory pouch; HG, hindgut; OV, ovariole.

fat body will often appear yellow when viewed through the cuticle. Dormant fat typically begins to accumulate in the anterior of the abdomen and appears to be closely associated with the saccular portion of the midgut. Thus, the hindgut and portions of the reproductive tract at the posterior of the abdomen are visible in weevils with a fat body status described as intermediate by Brazzel and Newsom (1959) (Fig. 2c). In weevils classified as fat, hypertrophy of the fat body has continued until all or nearly all of the other internal organs are obscured (Fig. 2d). As with the reproductive fat body, our experience indicates that status of the reproductive system influences the perception of fat body development. In addition, the

saccular portion of the midgut of an actively feeding weevil is frequently distended, and this organ is often difficult to distinguish from fat body when viewed through the cuticle. For these reasons, we recommend that fat bodies be rated only with the cuticle removed, and on occasion we find it necessary to remove the alimentary and reproductive tracts to assign a rating accurately.

Most reports of diapause studies indicate that fat body ratings of intermediate and fat were distinguished during data collection, but these ratings are rarely separated in presented results. The report of Wagner and Villavaso (1999b) is an exception, as they considered only the most developed fat bodies (rating

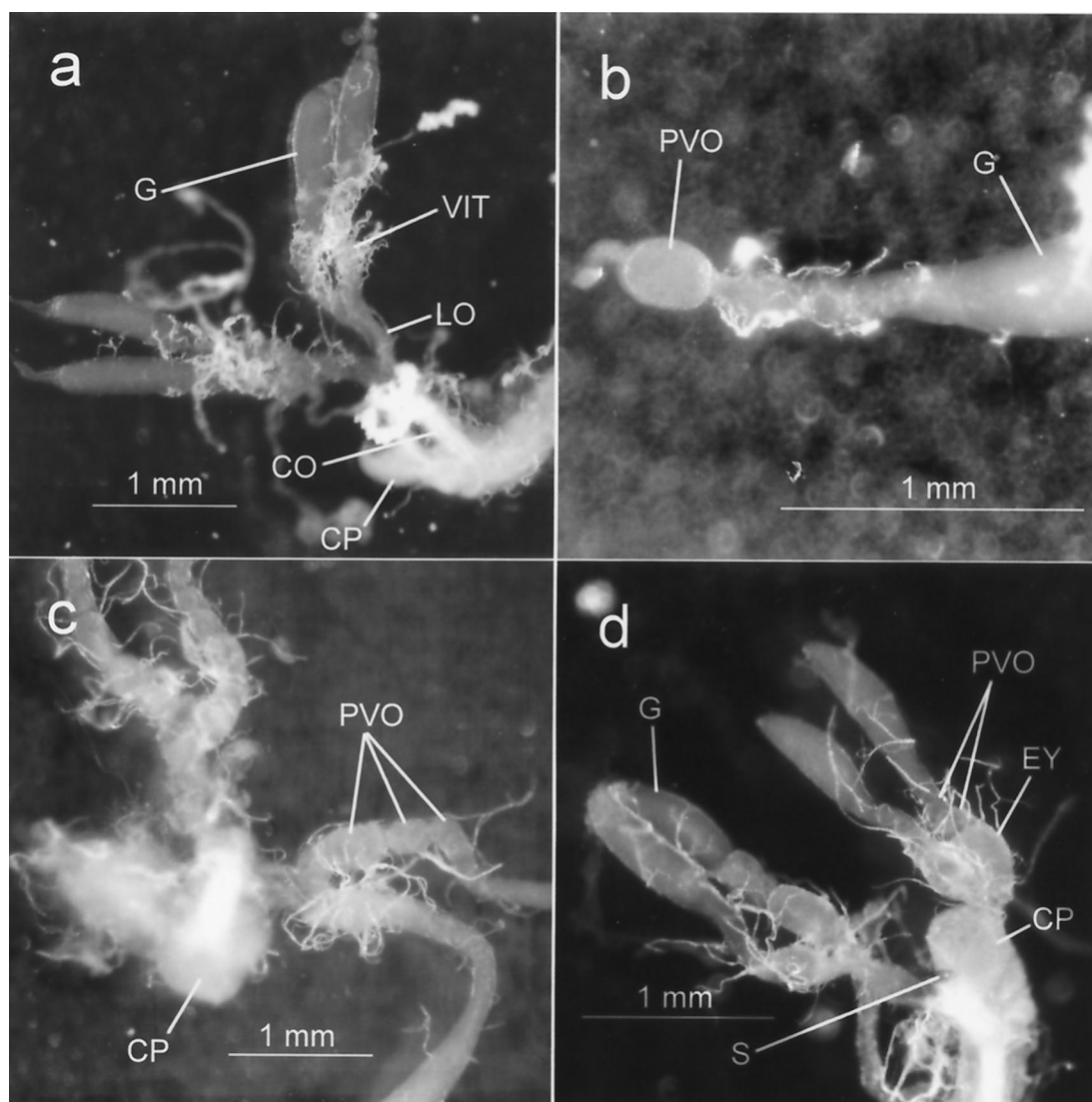


Fig. 3. Early stages of boll weevil ovarian development. (a) Prereproductive ovaries with no visible oocytes. (b) A previtellogenic oocyte typical of the earliest stage of reproductive development. (c) Ovaries containing numerous previtellogenic oocytes, but before visible signs of yolk uptake. (d) Ovaries in which the basal oocytes have initiated yolk uptake. CO, common oviduct; CP, copulatory pouch; EY, oocyte showing signs of early yolk uptake; G, germarium; LO, lateral oviduct; PVO, previtellogenic oocyte; S, spermatheca.

of 6 on a 0–6 scale) to be hypertrophied. Based on studies of host-free survival (D.W.S., unpublished data), and of overwintering survival and emergence (Westbrook et al. 2001), a classification of intermediate or fat represents the hypertrophy characteristic of diapause.

Female Reproductive Development. The female boll weevil contains two ovaries, and each ovary typically consists of two ovarioles (Burke 1959). On rare occasion, a female will be found with an ovary composed of one or three ovarioles (D.W.S., unpublished data). The ovaries are undeveloped at adult eclosion, with the germarium typically as long or longer than the vitellarium (Fig. 3a). This stage of ovarian develop-

ment corresponds to the N1 stage of Grodowitz and Brewer (1987). During this stage, the germaria may range in shape from elongate- and parallel-sided to slightly spindle shaped. The parallel-sided germaria tend to be smaller than those exhibiting a spindle shape. This stage of development is retained by many females entering diapause, and in those cases does not represent atrophy of the organs, as suggested by Brazzel and Newsom (1959), but rather a failure to develop after adult eclosion.

The first previtellogenic oocytes appear adjacent to the germaria in ≈ 1 (32.2°C) to 26 d (12.8°) (Spurgeon and Raulston 1998b). These first oocytes are difficult to detect unless the vitellaria are pulled taut, and often

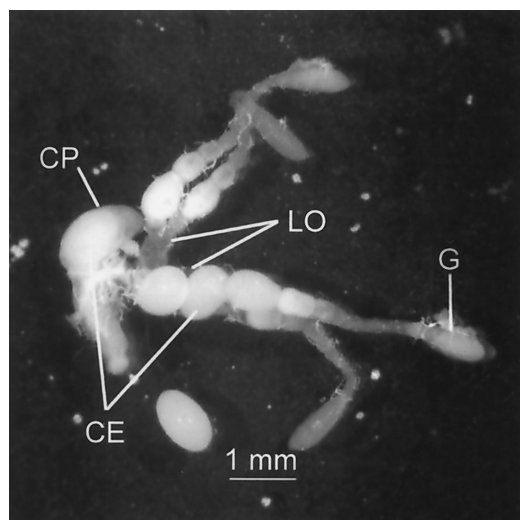


Fig. 4. Fully developed ovaries of a boll weevil, including chorionated eggs at the base of an ovariole, in a lateral oviduct, in the copulatory pouch, and removed from the reproductive tract. CE, chorionated egg; CP, copulatory pouch; G, germarium; LO, lateral oviduct.

require that the germarium and vitellarium be separated for positive identification (Fig. 3b). Additional oocytes (Fig. 3c) are produced rapidly after initial oocytes appear, provided dietary conditions continue to favor reproductive development. Diapausing females frequently contain ovaries with previtellogenic oocytes. The first oocytes with yolk accumulations (Fig. 3d) appear at weevil ages of 2 (32.2°C) to ≈ 38 d (12.8°C) (Spurgeon and Raulston 1998b). This earliest

accumulation of yolk is visually signified by increased opacity of the basal oocyte and presence of a large, apparent nucleus. For comparison, the N2 stage of Grodowitz and Brewer (1987) is a broader classification that includes a range of ovarian development from first previtellogenic oocyte to presence of well-developed oocytes with yolk, but that are not yet ready for ovulation.

The first chorionated eggs appear at the bases of the ovarioles in ≈ 3 d at temperatures $\geq 29.4^\circ\text{C}$ (Spurgeon and Raulston 1998b). These eggs are distinguished from adjacent oocytes by their uniform opacity and glossy surface (Fig. 4). Usually no more than a single chorionated egg occurs in each ovariole, but in unmated females or females denied an adequate oviposition substrate, chorionated eggs will occasionally accumulate in the vitellaria. Shortly after maturation, the egg will pass into the lateral oviduct, shedding the follicular epithelium in this process. In unmated females or when oviposition sites are limiting, we have observed accumulations of up to six eggs in each lateral oviduct (D.W.S., unpublished data). This stage of reproductive development encompasses the N3 (before ovulation) and P1 (after ovulation) stages of Grodowitz and Brewer (1987). Grodowitz and Brewer (1987) identified two additional parous stages (P2 and P3) based on the accumulation of follicular relics at the bases of the ovarioles, and related these stages to the numbers of eggs laid. However, these authors also acknowledged that misclassification of these stages may result from resorption or condensation of the relics. In addition, we have observed that relics sometimes pass into the lateral oviduct and are apparently voided during oviposition. For this reason, we ques-

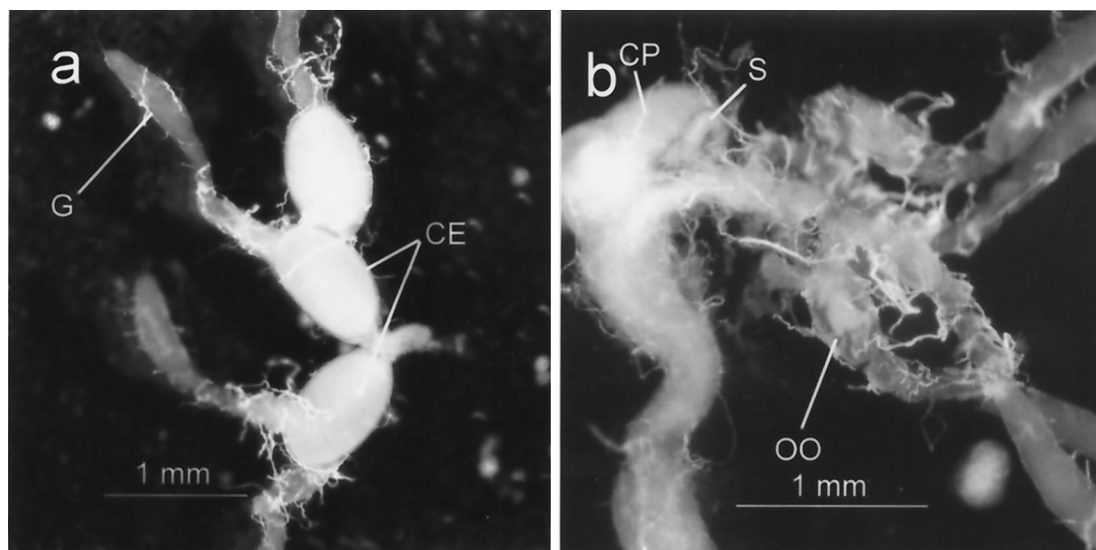


Fig. 5. Boll weevil ovaries typical of reproductive stasis or oosorption. (a) Ovaries in reproductive stasis with chorionated eggs, but exhibiting discontinuity in the progression of oocyte development. (b) Ovaries with oocytes exhibiting the condensation and flocculation of yolk typical of oosorption. CE, chorionated egg; CP, copulatory pouch; G, germarium; OO, oocyte undergoing oosorption; S, spermatheca.

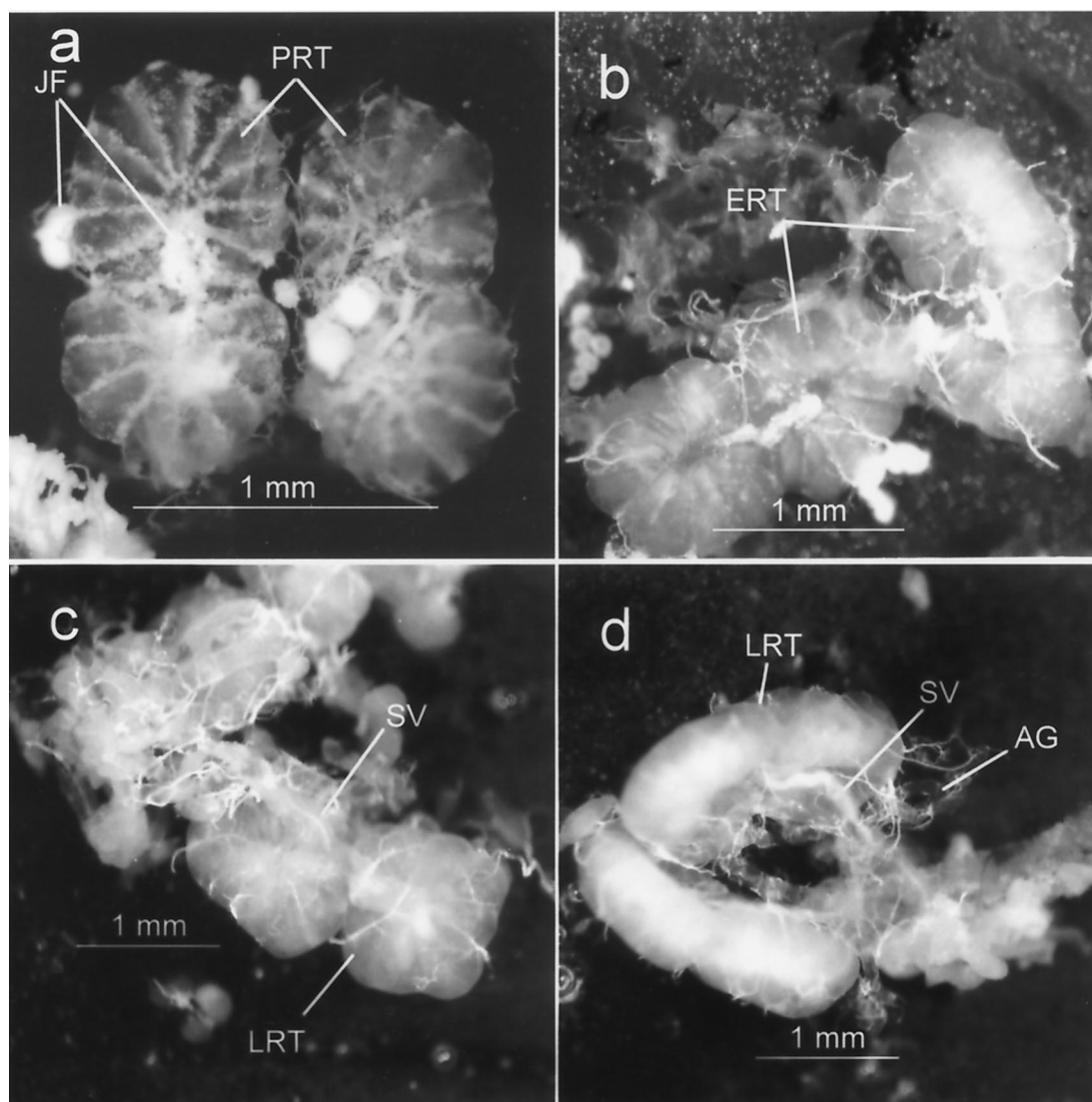


Fig. 6. Stages of male boll weevil reproductive development. (a) Undeveloped testes and adhering juvenile fat typical of a newly eclosed male. (b) Early-reproductive testes indicated by whitish streaks radiating from near the centers of the testis lobes, and with empty seminal vesicles. (c) Late-reproductive testes indicated by cloudiness in the periphery of the testis lobes, and with seminal vesicles refilling after mating. (d) Late-reproductive testes with seminal vesicles filled but not distended with sperm, and accessory glands poorly developed. AG, accessory gland; JF, juvenile fat; ERT, early-reproductive testis; LRT, late-reproductive testis; PRT, prereproductive testis; SV, seminal vesicle.

tion the utility of the relics for detecting or assessing the extent of prior oviposition.

Aside from the normal progression of female reproductive development, we have commonly observed two additional conditions of the ovaries in diet-switching and food-removal studies and from late-season field collections (D.W.S., unpublished data). The first of these conditions apparently represents a stasis in reproductive development (Fig. 5a). This condition is characterized by an obvious reduction in the complement of oocytes at ages beyond those required for full development of the ovaries and an apparent discontinuity in the progression of development of those

oocytes. Descriptions of ovaries similar to those illustrated in Fig. 5a were also provided by Wagner and Villavaso (1999a), although in our experience this condition is not always marked by the presence of chorionated eggs in the vitellaria or lateral oviducts. The second condition represents an actual cessation and reversal of reproductive development in which the yolk complement of oocytes is resorbed (Fig. 5b). Oosorption is visually characterized by unequal distribution of the yolk within the oocyte, giving the appearance of flocculation. In advanced stages (D.W.S., unpublished data), the remnants of the original yolk complement will be reduced to a small whit-

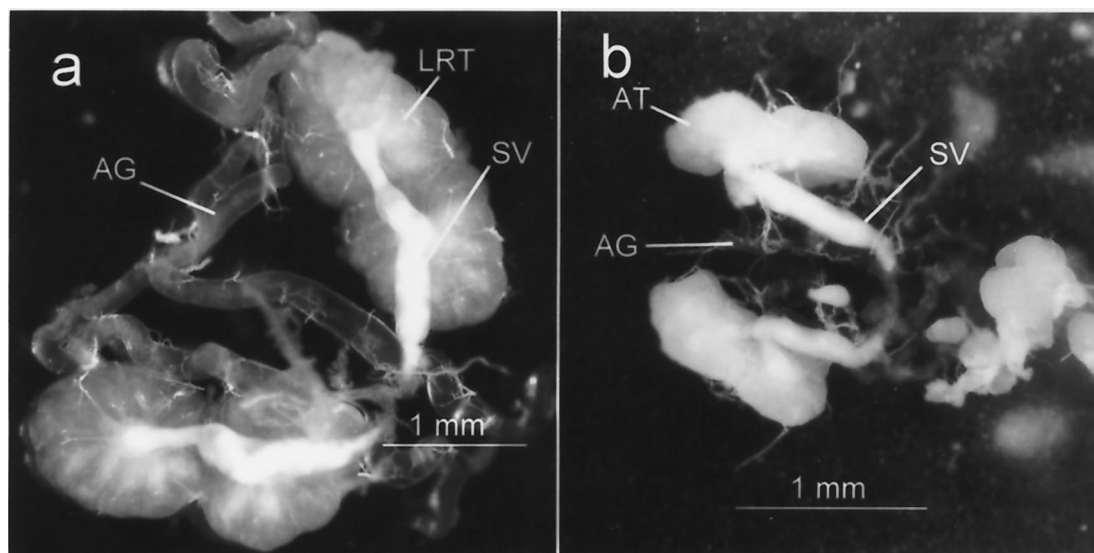


Fig. 7. Comparison of fully developed and atrophied male reproductive systems. (a) Late-reproductive testes with the seminal vesicles distended with sperm and well-developed accessory glands. (b) Atrophied testes opaque from external fat deposits, with filled seminal vesicles and poorly developed accessory glands. AG, accessory gland; AT, atrophied testis; LRT, late-reproductive testis; SV, seminal vesicle.

ish body near the center of the oocyte. Finally, the collapsed oocytes are condensed into amber- or brown-colored bodies that are easily mistaken for the follicular relics typical of ovulation. It is not uncommon for both the apparent stasis and evidence of oosorption to occur simultaneously. Chorionated eggs are apparently not so easily resorbed as oocytes, but we have observed highly vacuolated chorionated eggs in the vitellaria and lateral oviducts. In addition, we occasionally recover a collapsed chorion from the copulatory pouch, in which the chorionated egg was presumably crushed. The physiological and ecological implications of these conditions, which may occur in the presence or absence of hypertrophied fat bodies, have not been fully examined.

Male Reproductive Development. The male testes show no visible signs of internal development at the time of adult eclosion. The testicular lobes are translucent, and the sutures separating the septa may be marked by juvenile fat (Fig. 6a). The seminal vesicles are transparent at this stage of development and are typically difficult to discern with a dissecting microscope.

The first visible sign of reproductive development is the appearance of whitish streaks radiating from near the center of each testis lobe (Fig. 6b). This condition is closely followed by the development of opaque centers in the testis lobes. This stage was referred to as early reproductive by Spurgeon and Raulston (1998b), and it occurs at ages ranging from ≈ 1.5 to 2 d at temperatures $\geq 29.4^\circ\text{C}$ to 22 d at 12.8°C . During this stage of development, seminal vesicle condition may vary from empty, to beginning to fill, to filled but not distended. Seminal vesicles that are beginning to fill with sperm, whether from initial reproductive devel-

opment or after exhaustion of their contents from repeated mating, are characterized by a translucent to opaque mass of sperm near the center of the lumen of the organ (Fig. 6c). A distinguishing characteristic of seminal vesicles in the process of filling or refilling is the visible separation of the mass of sperm from the walls of the seminal vesicle.

Late reproductive testes occur at ≈ 3 –4 d ($\geq 29.4^\circ\text{C}$) to 35 d (12.8°C) after adult eclosion (Spurgeon and Raulston 1998b), and are characterized by opaque centers and clouding of the septa extending to, or nearly to, the outer extremities of the lobes (Figs. 6, c and d, and Fig. 7a). Seminal vesicle condition during this stage may vary from refilling (Fig. 6c), to filled but not distended (Fig. 6d), to distended with sperm (Fig. 7a). The distinction between seminal vesicles filled with sperm and those distended with sperm is subjective. In marginal cases, we have used the shape of the organ to aid in this classification, with distended seminal vesicles being distinctly club shaped until the distension becomes severe. The condition of the seminal vesicles depends largely on the frequency of mating activity; seminal vesicles distended with sperm are most often observed when males have been denied access to mates for several days.

In the diapausing male, the testes are typically light-yellow to nearly orange, reduced in size, and opaque from external fat deposits (Fig. 7b). Although Brazzel and Newsom (1959) reported the seminal vesicles of diapausing males lacked sperm, the condition of seminal vesicles associated with atrophied testes varies with the extent of mating activity (Spurgeon and Raulston 1998a). Although diapausing males held in single-sex groups normally contain seminal vesicles filled or distended with sperm, we have observed some

evidence of reduced seminal vesicle volume in association with atrophy of the testes (D.W.S., unpublished data). Observations provided by serial dissections in other studies (Spurgeon and Raulston 1996, 1998a; Spurgeon and Esquivel 2000) indicate the atrophied testicular state associated with diapause typically develops after the testes have progressed through the normal stages of reproductive development. The initial indication of pending atrophy is the accumulation of yellow fat in the sutures demarking the septa in late reproductive testes. As the testes diminish in size, their opacity increases because of continued fat deposition and/or the increased density of existing fat deposits resulting from a decrease in testicular surface area.

The accessory glands warrant special attention because Brazzel and Newsom (1959) provided descriptions of the glands associated with reproductive and diapausing males, while other investigators have largely ignored them. At adult eclosion, the accessory glands are transparent and inconspicuous. Although the accessory glands of diapausing males are also poorly developed (Fig. 7b), those of reproductive males are more variable in size and appearance than was reported by Brazzel and Newsom (1959). In males with late reproductive testes, accessory glands can vary from small and transparent (Fig. 6d) to greatly enlarged and containing a translucent gelatinous substance (Fig. 7a). Spurgeon (2001) and Young and Spurgeon (2002) found the condition of the accessory glands was related to rates of pheromone production. Also, unpublished observations during gut-emptying (Suh and Spurgeon 2001) and diet-switching studies indicated the condition of accessory glands changed rapidly when food was withheld. Thus, accessory gland condition is not a reliable criterion for distinguishing reproductive from diapausing weevils.

Although Spurgeon and Raulston (1998b) provide estimates of the time required for development to many of the illustrated stages of reproductive development, these stages of ovarian and testicular development are not accurate indicators of weevil age unless the diet has been closely controlled. The illustrations of ovaries in developmental stasis (Fig. 5a) and undergoing oosorption (Fig. 5b) accentuate this point. In addition, we have observed similar variation in the status of the testes (apparent early reproductive testes at advanced ages) during diet-switching studies, and from trap captures during the noncotton season (C.P.-C.S., unpublished data). Thus, an inconsistent diet or infrequent access to food can result in conditions of the reproductive organs that are inconsistent with the ages estimated by Spurgeon and Raulston (1998b).

Proposed Criteria for Distinguishing Diapause. Our proposed criteria for distinguishing diapause rely on assessments of the quantity and type of fat body and on the condition, but not the size, of the reproductive organs. We elect to disregard the criteria of Brazzel and Newsom (1959) regarding the length of the ovaries and diameter of the testes for several reasons. First, the sizes of adult weevils can vary dramatically

(Reardon and Spurgeon 2000), and this range in body size introduces the potential for considerable variation in the sizes of the reproductive organs irrespective of physiological status. Furthermore, when the fat body is present in abundance, the ovaries and testes must usually be cleaned of adhering fat before measurement. During this process, the ovaries are frequently irreversibly elongated and accurate measurement of their original length is not possible. Finally, Spurgeon and Raulston (1998a) devised a system to assess testis size in relation to the size of the weevil, similar to the system reported by Wagner and Villavaso (1999a). However, Spurgeon and Raulston (1998a) reported that although 96.2% of atrophied, opaque testes were classified as extra small, 11.5% of normal reproductive testes were also assigned to that size class. Thus, those authors concluded that the diapause and reproductive states could not be accurately distinguished on the basis of testis size.

Our criteria for distinguishing diapause require that the testes be atrophied or the ovaries undeveloped, and that the fat body be hypertrophied (dormant type of fat sufficiently abundant to rate intermediate or fat). In males, the testes should be small and opaque, or nearly opaque, with fat. In females, the ovaries may contain previtellogenic oocytes, but should not have developed to the stages containing oocytes with yolk. This distinction is based on the expected high metabolic costs of vitellogenin production and uptake, which would seem inconsistent with a state of dormancy intended to permit extended host-free survival. The distinction between our criteria and those reported in earlier studies lies mainly in our emphasis on characteristics of the hypertrophied fat body. In addition, we make no provisions for a classification of intermediate diapause, because this classification seems inconsistent with the endocrinological processes normally associated with the diapause state. At present, we do not include females with ovaries indicative of reproductive stasis or oosorption as diapausing, mainly because of a lack of experimental information to indicate their appropriate classification. However, we have captured successfully overwintered weevils with these characteristics in pheromone traps (C.P.-C.S., unpublished data), and continued experimentation may indicate their proper classification.

Our illustrations and accompanying descriptions provide more extensive guidelines for the classification of reproductive and diapausing boll weevils than were previously available. We recognize that many aspects surrounding boll weevil dormancy remain unresolved and that other investigators may choose to employ diapause criteria other than those we propose. At minimum, the results we present provide a baseline for comparison in the description of alternate criteria and should serve to clarify these descriptions in future reports.

Acknowledgments

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